Objectives

- Recognize the use and challenges of touch preparations in small biopsy specimens from common and uncommon entities.
- Understand the key cytomorphological features and utility of ancillary studies in small biopsy evaluations to make more specific diagnoses or to help guide treatment.
- Evaluate the differential diagnosis for different lesions in touch preparation cytopathology that can help in the triage of the specimen.

Outline

- Introduction
  - Advantages & Disadvantages

- Case-Based Approach
  - Touch Preparations in different locations

- New Applications
  - Touch Preparations in Clinical Trials

- Conclusion

Introduction

- Increase in use of small biopsies for the diagnostic evaluation of lesions
  - Improvement in needles available
  - Less expensive & safer alternative to surgical resection
  - Pressure to do more with less
  - Validation of many new tests on tissue specimens first

Use of Touch Preparations

- Touch/Imprint preparations have been used for years
  - Intraoperative consultations
  - ROSE of small tissue samples
  - Other: Testis/fertility, Tzank test, Imprints for ancillary studies

- Methods
  - Imprint
  - Drag
  - Roll
  - Touch & Pick
Piece of tissue or core is placed on a glass slide and gently dragged along the surface of the slide.

A tissue core is lightly rolled on a glass slide. Solid, firm cores (e.g. bones) are easy to roll.

A tissue/core is gently touched on to the surface of a clean glass slide and then picked up again without dragging it.

TP Methods

- **Imprint**: Pressing a glass slide on to a large tissue specimen or resected organ that is freshly cut for evaluation.
- **Drag**: Piece of tissue or core is placed on a glass slide and gently dragged along the surface of the slide.
- **Roll**: A tissue core is lightly rolled on a glass slide. Solid, firm cores (e.g. bones) are easy to roll.
- **Touch & Pick**: A tissue/core is gently touched on to the surface of a clean glass slide and then picked up again without dragging it.

**2016**

Survey of CAP non-gynecologic cytology education program.
- Preparation of touch imprints performed by cytotechnologists (49.7%), pathologists (45.4%), and less often by laboratory aides (20%).
- Techniques used to prepare a touch imprint included touching the CNB on the slide (50.5%), rolling the CNB on a slide (45.6%), and rarely (3.1%) a crush preparation.

**Technique**

- **TP procedure can affect**:
  - Number of slides made
  - Stop making TPs if lesional material is obtained
  - Technique used to prepare TP
    - Imprint, Drag, Length of Drag
    - Depends on personnel preparing TP
    - Preserve sterile CNB needle or not
  - Quality of TP for ROSE
    - Too thick or too crushed
  - DNA content of CNB
    - If too vigorous, 25% decrease in DNA content, higher with longer drag and material loss

**2015**

- Diagnostic accuracy was equivalent for all TPs.
- Vigorous TPs (e.g. 2cm drag) contain substantial fraction of CNB cellularity & limit CNB DNA content.

### Table 1. Cellularity Discrepancy According to Sample Site

<table>
<thead>
<tr>
<th>Sample Site</th>
<th>Discrepant Cellularity</th>
<th>No. of Cases With More Tumor Cells in TP</th>
<th>No. of Cases With More Tumor Cells in CNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft tissue</td>
<td>Discrepant Cellularity</td>
<td>25/64(39%)</td>
<td>6</td>
</tr>
<tr>
<td>Bone</td>
<td></td>
<td>18/32(56%)</td>
<td>7</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>24/52(46%)</td>
<td>16</td>
</tr>
<tr>
<td>Vascular organ</td>
<td></td>
<td>8/12(67%)</td>
<td>3</td>
</tr>
<tr>
<td>Lymph node</td>
<td></td>
<td>5/9(56%)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>84/100(84%)</td>
<td>30</td>
</tr>
</tbody>
</table>

**Cellularity Score**:
- Cellularity Score: Most cellular slide of the case was assigned score 0-3 for cellularity:
  - 0: No tumor cells
  - 1: 1-10 tumor cells
  - 2: 11-50 tumor cells
  - 3: >50 tumor cells

**Cellularity Discrepancy**:
- Cellularity Score differential of 2 or more

Why use Touch Preparations?

- Allow for ROSE of CNBs
- Less potential for tissue loss, compared with frozen section
- Maximize diagnostic yield
- Minimize cases with insufficient material for ancillary studies
- Potentially more tissue, especially for lesions that are difficult to sample with FNA
- Avoid repeat procedures (and save health care dollars)

Other factors affecting TPs

- ROSE service availability
- Preceding FNA for needle placement
- Location
- Tumor type
- Presence or absence of fibrosis
- Size of needle
- Concordance/Discordance with CNB
- Need to triage during procedure: Lymphomas, Infections, etc

Table 2. Findings in Cases Showing the Presence of Tumor Cells in Either TP or CNB Only (N = 43)

<table>
<thead>
<tr>
<th>Organ Site</th>
<th>No. of Cases</th>
<th>Tumor Cells in TP</th>
<th>Tumor Cell in CNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft tissue</td>
<td>16 (27.6%)</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Lung</td>
<td>15 (61.5%)</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Bone</td>
<td>7 (36%)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Vestibular organ*</td>
<td>4 (19%)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lymph node</td>
<td>1 (5%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>43 (100%)</td>
<td>15</td>
<td>28</td>
</tr>
</tbody>
</table>

15 (1.4%) cases with depletion of tumor cells from TP/processing
9 (2.6%) lung cases with depletion of tumor cells from TP/processing

Monaco S et al (UPMC), Diagn Cytopathology, 2019 (in press)
To touch or not?

- Diagnostic Concordance Rate of TP & CNB: High (88-95%)
- Advantages of TP (Support of TP with CNB):
  - Ability to evaluate tissue for diagnosis with less tissue loss than frozen section
  - Ability to direct intra-procedural care: sample more/less, sample different lesion, triage
- Disadvantages of TP (Support of CNB without TP):
  - Potential for cell depletion -> less material for ancillary studies, more likely with vigorous touch preparations
  - Artifacts: Streaking & crushing of cells, US gel contamination & thick material


To touch or Not to touch? How do you touch?

Touch Preparations: Pearls

- Prepare TP along the short axis of the slide
- CNB should not be dragged for >1 cm along the slide surface
- Minimal tissue manipulation is desirable
- Slide choice: Uncoated slides may minimize excessive tissue loss, opposed to coated slides
- For delicate CNBs, consider alternate methods or avoid touching (e.g. 20-21G CNBs, necrotic CNBs)
- Cost saving measures: To reuse the needle for the CNB, use a sterile needle or cap to pick the core off the needle

Pantanowitz, Xing, & Monaco. Atlas of Touch Preparation Cytopathology, 2018

Case History

- 82 year old woman with incidental well-circumscribed lesion in right upper lobe of the lung.

- Prior CTG FNA showed features of a pulmonary hamartoma, but lesion was growing on imaging.

- Procedure: CTG FNA & CNB with TP
Case Diagnosis

- **Final Diagnosis:**
  - Satisfactory for Interpretation
  - Positive for neoplasm
  - Salivary gland-type tumor, favor Epithelial-Myoepithelial Carcinoma.

- **Challenges:**
  - Biphasic lesions in the lung: not always hamartoma
  - Salivary gland-type tumors: primary versus metastatic
  - Lung tumors that do not fall into SCLC vs NSCLC are challenging

Follow-up

- No primary salivary gland lesion identified on CT-PET scan.
- Lobectomy showed a well-circumscribed, lobulated lung lesion grossly.
- Resection showed:
  - Carcinoma ex Pleomorphic adenoma
  - Carcinomatous component was an EMC (Epithelial-Myoepithelial carcinoma)

Carcinoma ex Pleomorphic Adenoma in Lung

- Rare in the lung, but arises from the bronchial glands
- Must exclude a head and neck primary
- Considered a low-grade malignancy with long interval to recurrence or metastasis
- Most common carcinomas in this setting:
  - Poorly differentiated adenocarcinoma
  - Salivary duct carcinoma
  - Epithelial-myoepithelial carcinoma
- Gross: well circumscribed, pushing border in an endobronchial location

Differential Diagnosis

- **Benign:** Granuloma, Amyloidoma
- Hamartoma
- Mesenchymal tumor (e.g. solitary fibrous tumor, sarcoma)
- Metastatic spindle cell tumor with myxoid change (e.g. GIST)
- Salivary gland-type tumor
  - Primary (arising from the bronchial glands) vs. Metastatic
  - Benign (pleomorphic adenoma) vs. Malignant (epithelial-myoepithelial carcinoma)
  - Variable subtypes: pleomorphic adenoma, epithelial-myoepithelial carcinoma, adenoid cystic carcinoma, mucopidermoid carcinoma, basal cell neoplasm
- Primary lung carcinoma with desmoplastic stroma or mucin (Adenocarcinoma, Basaloid squamous cell carcinoma, Carcinosarcoma)

Pulmonary Hamartoma

- Scant cellularity
  - Due to dense nature of the lesion
  - Rubber eraser-like effect
- Clean Background
  - No necrosis or inflammation
- Reactive bronchial cells
- Cartilaginous or Fibromyxoid fragments (metachromatic)
- Recurrent clonal rearrangements of HMG(Y) gene on chr.6p21
Take Home Messages

- Pulmonary hamartomas typically do not grow rapidly.
  - Increased growth on serial imaging is a RED flag.
- Think of SGTTs in the lung when you see a biphasic tumor with chondromyxoid material and basaloid or myoepithelial-type cells.
  - Atypical features to look for in a fibromyxoid lesion in the lung: high cellularity, atypia, bilayered glandular structures, and lesional growth
- Although SGTTs can occur as a primary in the lung (from the bronchial glands), a metastatic tumor should be excluded.
- Limitation of FNA in some lesions: CNBs of the lung can be helpful in certain situations

A Touch of Kidney Biopsies
Renal/Adrenal biopsies: Then & Now

**Historically**
- Less advanced imaging
- Lack of confidence by urologists due to high FN rates for renal biopsies/FNA
- Belief that a biopsy would not alter patient care
- Fear of complications: hemorrhage, needle tract seeding, infection

**Currently**
- More advanced imaging
- Studies showing high sensitivity & specificity
- Complications only rare (0-1.3%)
- Biopsy is considered safe and cost-effective
- Helps avoid surgery for non-surgical diseases (infection, lymphoma, metastasis)

Recent Developments
- Increased utilization of imaging modalities (US, CT, MRI)
- Increased detection of small solid renal & adrenal masses
- 8-27% of surgically resected solid renal masses were benign
  - As size of lesion decreases, chance of benign lesion increases
- Biopsies have been shown to change clinical management in over 50% of patients in which a biopsy is performed.
- Thus, growing need for small renal mass biopsies when imaging is inconclusive.

Indications for Kidney FNA/CNB
- **Conditions where a radical nephrectomy is contraindicated**
  - Unresectable RCC, Metastases, Poor surgical candidates with comorbid conditions, or desire for non-surgical treatment (e.g. ablation)
- **Lesions with indeterminate radiological findings**
  - Atypical cysts, Small solid lesions (DDx: fat free AML, oncocytoma)
- **ASCO Clinical Practice Guidelines:** Consider biopsy if the results will alter treatment (partial nephrectomy vs thermal ablation vs radical nephrectomy)

Note: Less than 10% of adult renal lesions typically undergo FNA/core biopsy

Case History
- **75 year old man with large renal mass**
  - Indeterminate & ill-defined by imaging. Hypovascular.

  **Procedure:** CTG FNA and CNB with TP

Schmidbauer J et al, Europ Urol 2008
Volpe A et al, Arch Urol 2009

Case Diagnosis

- **Final Diagnosis:**
  - Satisfactory for Interpretation
  - Positive for malignant cells
  - Large B-cell Lymphoma, CD20-positive.
- **Flow on FNA:** Positive for B-cell Lymphoma with germinal center phenotype.
- **FISH:** Positive for BCL6 gene rearrangement, negative for BCL2 and MYC gene rearrangements.
- **Nephrectomy cancelled.**
- **Challenges:**
  - Diagnostic Pitfall: Normal renal elements & identifying lymphoid cells

Normal Elements in Kidney

- **Glomeruli**
- **Proximal tubular cells**
- **Distal tubular cells**
- **Benign cells from needle tract**

**Beware on touch preparations and aspirates!**
These cells could make one falsely assume there is diagnostic material from a renal mass.

**Glomeruli**

- **Cellular**
- Cells not evenly distributed, dense at the center than at the periphery.
- Capillary loops
- **DDx:** Papillae of papillary RCC & other tumors, Granulomas

**Proximal Tubular Cells**

**Oncocytoma**

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximal Tubular Cells</strong></td>
<td><strong>Oncocytoma</strong></td>
</tr>
<tr>
<td>Lower cellularity</td>
<td>Higher cellularity</td>
</tr>
<tr>
<td>Uniform, round, bland nucleus</td>
<td>Variable nuclear size and shape</td>
</tr>
<tr>
<td>Small nucleolus</td>
<td>Variable nucleolus</td>
</tr>
<tr>
<td>Lack of well-defined cell borders</td>
<td>Well-defined cell borders</td>
</tr>
<tr>
<td>Granules appear to spill out of cells</td>
<td>Granules confined within cell borders</td>
</tr>
<tr>
<td>Other background findings: glomeruli, distal tubular cells</td>
<td>Typically a single population without glomeruli or other cells</td>
</tr>
<tr>
<td>Non-diagnostic/Benign</td>
<td>Diagnostic/Neoplastic</td>
</tr>
</tbody>
</table>
Kidney CNB: Dx or Non-Dx?

Typically a lesional biopsy if:
1. Fragmented
2. Notable transition present

False positives in Kidney FNA/Core Bx

- Xanthogranulomatous pyelonephritis
- Angiomyolipoma
- Benign hepatocytes
- Benign tubular cells
- Benign adrenal cortical cells

- Benign mimics of malignancy can contain atypical cells, but they are usually few in number or the sample itself is hypocellular.
- Most hypocellular kidney aspirates, therefore, should not be diagnosed as positive even if they contain some atypical cells.

Renal Cell Carcinoma

B-cell Lymphoma

Xanthogranulomatous Pyelonephritis
Take Home Messages

• Beware of benign cellular components on TP & FNAs of Renal Masses

• Accurate identification of components on TP or FNA can help triage the specimen to avoid insufficient or non-diagnostic results

• Maximizing use of pre-operative small biopsies for indeterminate renal masses can prevent surgery or direct the best management.

A Touch of Retroperitoneal Biopsies

Retroperitoneal small biopsies

• Could be anything
  • Correlate with radiology to see where it is arising from or near

• Think of all possibilities:
  • Soft tissue lesions, renal/adrenal lesions, lymphoid lesions, metastases, & histiocytic lesions

• Think of uncommon things in certain scenarios:
  • Transplant patients: Infections & EBV+ related neoplasms

Case History

• 40 year old woman with history of inflammatory skin lesions diagnosed in 2013, now found to have a 4.6 cm soft tissue mass near the right psoas muscle, “hairy kidney”, and FDG-avid sclerotic bone lesions.

• Procedure: CTG FNA and CNB with TP performed of the soft tissue mass.
Case Diagnosis

- Soft tissue infiltrate, Pelvic, CT-guided FNA:
  - Less than optimal- scant cellularity.
  - Atypical cells present.
  - Atypical histiocytic proliferation.

- Soft tissue infiltrate, Pelvic, CTG CNB with TP:
  - Histiocytic proliferation, compatible with Erdheim-Chester Disease.

Follow-up: Treated with methotrexate and Imuran (Azathioprine) with partial clinical resolution of skin lesions and bone lesions, but required stenting of ureter due to fibrosis.

Erdheim-Chester Disease (ECD)

- Non-Langerhans cell histiocytosis
- Polysostotic sclerosing histiocytosis
- Onset: Middle adult age (approximately 50 years of age)
- Clinical symptoms: Bone pain (most frequent), Back pain, Renal pain/dysfunction, Exophthalmos with yellow bumps on eyelids, problems with coordinated movements, skin lesions
- Locations: bone, retroperitoneum ("hairy kidney"), & CNS
- Disease course: Ranges from single system disease (focal lesions, bone only) to multisystemic & fatal disease (multiple visceral organ involvement)
  - Prognosis appears to be worse than other histiocytoses, so important to diagnose

Non-LCH disorders typically include 3 groups:
1. Primarily cutaneous involvement only (JXG)
2. Cutaneous and systemic involvement
3. Primarily extracutaneous involvement (ECD)
ECD: Cytological Findings

- **Cytology:**
  - Lipid-laden foamy histiocytes
  - Touton-type giant cells
  - Bland spindle cells/fibrosis
  - No nuclear grooves
  - No eosinophils
  - No Birbeck granules on EM
  - No cytological atypia to suggest a histiocytic or dendritic cell sarcoma

Purgina B et al, Cytojournal 2011

ECD: Histological Findings

- **Histology:**
  - Xanthogranulomatous infiltrate
  - Marked fibrosis

ECD: Immunohistochemical Findings

- **IHC:**
  - + CD68, CD163, Factor XIIIa, fascin, and +/- S100
  - Xanthogranuloma phenotype CD163/CD68/fascin/FXIIIa
  - - CD1a, Langerin
  - No Birbeck granules

ECD: Molecular Findings

- **Molecular:**
  - BRAF V600E mutations occur in 57-69% patients with isolated LCH and in 54-82% patients with isolated ECD
  - BRAF Mutation occurs in LCH & ECD
  - "Mixed Histiocytosis" cases do exist: cases with overlap between LCH/ECD
  - No BRAF mutation in non-LCH histiocytosis (except for ECD)
    - e.g. Rosai-Dorfman disease, cuboidal juvenile xanthogranuloma, histiocytic sarcomas, xanthoma disseminatum, interdigitating dendritic cell sarcoma, & necrobiotic xanthogranuloma
  - Neoplasms that are positive for **BRAF V600E**
    - Metanephric adenoma of the kidney
    - Papillary craniopharyngioma
    - Pleomorphic xanthoastrocytoma
    - Ameloblastoma
    - Langerhans cell histiocytosis
    - Erdheim Chester Disease
    - Hairy cell leukemia-classic (Not in HCL-variant)
    - Papillary thyroid carcinoma
    - Colonic adenocarcinoma
    - Malignant Melanoma

ECD: Molecular Findings

- **Molecular:**
  - *BRAF* V600E mutations occur in 57-69% patients with isolated LCH and in 54-82% patients with isolated ECD
  - *BRAF* Mutation occurs in LCH & ECD
    - "Mixed Histiocytosis" cases do exist: cases with overlap between LCH/ECD
  - No *BRAF* mutation in non-LCH histiocytosis (except for ECD)

ECD: Differential Diagnosis

- Non-diagnostic/non-specific findings
- Non-neoplastic xanthogranulomatous proliferation
- Fat necrosis
- Hemophagocytic syndromes
- Other Histiocytoses
  - LC
    - Non-Langerhans cell histiocytosis
    - Clear cell type neoplasms (e.g., Metastatic RCC)
    - Sarcoma (e.g., Lipoascroma)
    - Spindle cell proliferation (e.g., Fibromatosis)
**Take Home Messages**

- **Histiocytic lesions/neoplasms can be challenging**
  - Correct classification requires correlation with the clinical features
  - Xanthogranulomatous lesions with fibrosis may be scant on FNA/TP or mistaken as normal findings on CNB

- **Utility of ancillary testing**
  - IHC +/- Molecular studies
  - Exclude other types of histiocytoses and non-histiocytic neoplasms
  - The presence of the BRAFV600E mutation in any XG-type lesion should prompt a work-up for ECD
Diagnostic Challenges with Small biopsies in histiocytic & fibrotic lesions

• **Acquisition Challenges**
  - Sampling issues due to focal or patchy disease & may need to biopsy multiple sites to reach correct diagnosis
  - Need to correlate pathology findings with radiology

• **Interpretation Challenges**
  - Be careful not to dismiss findings as NonDx or Negative
  - Need to have suspicion in order to initiate IHC panel

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Cautionary Note

• Don’t fight with the radiologist/clinician!

• There really may be something in the CNB if TP is scant.

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Touch Preparations in Clinical Trials

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Reasons for Requesting ROSE for Clinical Trials

• **Confirmation of viable lesional material**

• **Special handling of tissue**
  - Need for fresh, unfixed tissue with minimal cold ischemia time
  - Submission of material with specialized processing instructions

• **Optimizing Informative Results**
  - Avoiding FN results by testing of normal tissue
  - Avoiding Non-diagnostic results & repeat procedures

• **Improving Patient Care**
  - Avoiding CNB in risky cases by determining if FNA is sufficient
  - Minimizing discomfort and repeat procedures for cancer patients (esp those with poor response to therapy & multiple prior procedures)

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**TABLE 1. Clinical Trial Information Needed When Coordinating Pathology Involvement**

- Patient name and additional identifier (e.g., date of birth, medical record number)
- Site of biopsy
- Indication for biopsy
- Study protocol
- Special instructions (e.g., tissue processing, handling)
- Warnings for TMA
- Special requirements for tissue processing
- Instructions for sending (if not being entered into the laboratory information system as a diagnostic specimen)

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Manzo J et al (UPMC), Cancer Cytopathology, 2018;126:481-89.
Bad TP, DQ stain: Problematic (US gel)

Metastatic lung adenocarcinoma

FNA Pass 1, DQ stain

Metastatic urothelial carcinoma

FNA Pass 2, DQ stain

Pass

CB, H&E

CNB, H&E

75 yo F with Hx Breast Carcinoma.

Primary Invasive Ductal Carcinoma

New Primary Myxofibrosarcoma

Conclusions

• Minimally invasive biopsies have changed the way that many lesions are approached

• Cytopathology laboratories have had to adjust to this new demand

• Diagnostically Challenging: ROSE of small biopsy TPs can be challenging, but critical for allocating the small biopsies appropriately

• New Applications: ROSE of TPs from small biopsies to maximize yield for clinical trials or research testing

Thank you!